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Ecosystem responses to increased organic carbon concentration: comparing results based on long-term monitoring and whole-lake experimentation

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Abstract

Recent increases in terrestrial dissolved organic carbon (DOC) concentrations in northern inland waters have many ecological consequences. We examined available data on carbon cycles and food webs of two boreal headwater lakes in southern Finland. The basic limnology of Lake Valkea-Kotinen was monitored over the past 25 years while the lake has undergone browning and DOC increased from ca. 11 to 13 mg C L^{-1}. Pronounced changes in the early 2000s represent a regime shift in DOC concentration and color. Lake Alinen Mustajärvi was manipulated for two years by additions of labile DOC (cane sugar) raising the DOC concentration from ca. 10 to 12 mg C L^{-1}. The two different approaches both revealed increased concentrations and efflux of carbon dioxide (CO_2) from the lakes, thus, net heterotrophy, and changes in the pelagic community structure following an increase in DOC concentration. In Valkea-Kotinen browning caused a decline in phytoplankton primary production, which was reflected in retarded growth of young (1-2 year old) perch. The growth of young perch varied more in the experimentally manipulated lake, and the results suggested the importance of a pathway from labile DOC via benthic invertebrates to perch. Although provided with this extra resource, the food chain based on DOC proved inefficient. Long-term monitoring of lakes in pristine catchments is crucial for revealing how freshwater ecosystems respond to climate and/or atmospheric deposition induced changes, such as browning. Short-term whole-lake manipulation experiments can provide insights into the processes behind the patterns and trends demonstrated in long-term studies.

Keywords (3-6)

Boreal lakes, browning, terrestrial organic carbon, food webs, lake metabolism, CO_2 flux

Introduction

During recent decades increased concentrations of terrestrial dissolved organic carbon (DOC) and associated water browning has been reported in many lakes in the northern hemisphere (e.g. Jennings et al. 2010, Couture et al. 2012). This phenomenon has been related to various processes including recovery from earlier acidification, increasing precipitation and runoff, climate warming connected with increase in vegetation cover and prolonged frost-free period of soils as well as land-use changes (Vuorenmaa et al. 2006, Monteith et al. 2007, Lepistö et al. 2014, Finstad et al. 2016). In addition to colored terrestrial DOC, increased concentration of iron can also contribute to water browning (Krizberg and Ekström 2012, Weyhenmeyer et al. 2014). The future impact of browning on boreal lakes is difficult to predict, because there are several potential impacts on lake properties and processes (Solomon et al. 2015, Creed et al. 2018). Firstly, the increased water color reduces light penetration affecting both light quantity and quality, which in turn may reduce both pelagic and benthic primary production as well as the balance between these processes (e.g. Karlsson et al. 2009). Secondly, terrestrial organic matter entering lakes provides additional substrate for bacteria (Tranvik 1988, Tulonen et al. 1992), increasing production of CO_2 and shifting lake metabolism towards net heterotrophy (e.g. del Giorgio and Peters 1994, Sobek et al. 2003, Hanson et al. 2003, Ask et al. 2009). Such changes may also have implications for food web structure and energy transfer efficiency (Jansson et al. 2007, Carpenter et al. 2015). Thirdly, terrestrial organic matter loading will also bring nutrients such as phosphorus (P) and nitrogen (N), potentially available for primary producers (Jones 1992, Seekell et al. 2015). Disentangling the consequences of these parallel potential effects of terrestrial DOC increase has been extremely challenging, and will require different but complementary approaches.

Three basic approaches have been used to investigate the effects of lake browning and DOC increase. Spatial surveys comparing the properties of lakes that currently exhibit different

concentrations of DOC assume that future temporal change will reflect present spatial variation, but the assumptions underpinning this "space-for-time" approach are likely to be at best weak and at worst invalid (e.g. Clark et al. 2010, Solomon et al. 2015, Kelly et al. 2016). Monitoring the properties of particular lakes over a long time span can reveal the impact of browning on lake properties, but the generality of the findings will be uncertain. Moreover, neither of these approaches can properly identify the mechanisms underpinning the patterns revealed: for that experimentation is required. However, experiments using mesocosms to investigate impact of DOC on lake properties (e.g. Arvola et al. 1996, Faithfull et al. 2012, Jones and Lennon 2015) suffer from lack of realism or relevance, because mesocosms only represent a part (usually the open water pelagic) of an entire lake ecosystem, are hydrologically isolated from the critical terrestrial loadings from the catchment, and only yield meaningful results for very short (days-few weeks) time periods. Hence, experimental investigation of the effects of DOC increase are needed at the whole-lake scale, which is logistically challenging and has only rarely been attempted (e.g. Blomquist et al. 2001, Peura et al. 2014, Kelly et al. 2016).

Here we use data available from two small boreal headwater lakes in southern Finland to compare how an increase in DOC concentration affected their carbon cycles and food web structures. The data reflect two different approaches: long-term-monitoring and whole lake manipulation experiments. Since 1990 Lake Valkea-Kotinen has been monitored for several limnological variables, primary production and the food web up to fish. During this period the DOC concentration of the lake increased from ca. 11 to 13 mg C L^{-1} and water color values increased from ca. 100-150 to 150-200 mg Pt L^{-1}, whereas the mean daily primary production in the photic layer (0-1 m) declined from ca. 130 to 70 mg C m^{-3} d^{-1} (Arvola et al. 2014). Lake Alinen Mustajärvi was experimentally manipulated by increasing the epilimnetic DOC concentration from around 10 to around 12 mg L^{-1} for two ice-free periods by adding

cane sugar into the lake. The sugar addition was intended to increase the availability of labile bacterial substrate without affecting either light penetration or nutrient concentrations, and hence to isolate and study one of the parallel potential effects of lake browning. In addition, the fate of the added DOC could be traced in organic and inorganic carbon fractions and onwards through the pelagic and benthic food webs up to fish (Peura et al. 2014, Jones et al. 2018) based on the $\delta^{13}C$ differences between cane sugar, originating from a C4 plant, and that in local terrestrial organic carbon from C3 plant origin. Here we (1) evaluate whether the experimental whole-lake manipulation results can help us to better understand the long-term ecosystem changes in response to changes in color and DOC concentrations, and (2) discuss the relative merits of different but complementary approaches in the study of lake carbon cycles and food webs.

Methods

Lake Valkea-Kotinen (henceforth VK) in southern Finland (61°15'N; 25°04'E) is a small boreal headwater lake located on a pristine old-forest nature conservation area (Ruoho-Airola et al. 2014). The lake and its catchment are part of the Finnish Long-Term Socio-Ecological Research network (FinLTSER) and have been continuously monitored for over 25 years (see Rask et al. 2014). This lake has an area of 0.04 km^2, a catchment area of ca. 0.3 km^2, a maximum depth of 6.5 m and an estimated volume of $103x10^3$ m^3. The whole-lake manipulation was conducted in Alinen-Mustajärvi (henceforth AM), another small boreal headwater lake just 4 km from VK (61°12'N; 25°06'E). During the study years no forestry operations were carried out in the catchment area. The lake has an area of 0.007 km^2, a catchment area of ca. 0.04 km^2, a maximum depth of 6.5 m and an estimated volume of $31x10^3$ m^3. Both lakes are typically ice-covered from around late November to late April, and during summer develop a very steep and stable stratification with an epilimnion depth of around 2 m and an anoxic hypolimnion. Littoral macrophyte vegetation is highly restricted in

both lakes due to the poor light penetration into these humic lakes, however, floating littoral vegetation mats with associated periphyton can be highly productive in such lakes (Vesterinen et al. 2016) and can harbour abundant macroinvertebrates. Both lakes contain reproducing populations of European perch (*Perca fluviatilis*) and of Northern pike (*Esox lucius*). Further information about the characteristics of the two lakes can be found elsewhere (e.g. Arvola et al. 2014, Rask et al. 2014, Peltomaa 2013, Peura et al. 2014).

In AM, water chemistry and carbon cycle variables were measured during one open water period (2007) before cane sugar was added. During the open water periods of 2008 and 2009, 66 kg of cane sugar was added monthly to the epilimnion (from May to October), equivalent to a concentration of 2 mg C L^{-1} of DOC or a mean daily loading of 0.07 mg C L^{-1} to the epilimnion (22 g C m^{-2} yr^{-1}). The carbon addition was made assuming the proportion of labile DOC in the lake to be around 15 % of the total DOC (e.g. Tranvik 1998), and represents an increase in labile DOC to the level expected for the fourth quartile of boreal lakes according to the Nordic Lake Survey (Henriksen et al.1998). Prior to the addition to the lake, the cane sugar was dissolved in lake water and the sugar solution was then distributed evenly across the lake surface from a rowing boat.

Most basic data used in this paper have been reported previously, and the methods used to collect the data have been described in detail elsewhere and will not be repeated here. For VK monitoring see Vuorenmaa et al. (2014) for chemistry, Arvola et al. (2014) for plankton metabolism, Peltomaa et al. (2013) for phytoplankton, Lehtovaara et al. (2014) for zooplankton and Rask et al. (2014) for fish. Respective methods used in AM studies are described by Peura et al. (2014) and Jones et al. (2018). Only the results of variables measured during the summer stratification period (June – September) in both lakes using the same methods and the same instruments at Lammi Biological Station are compared.

For detecting possible abrupt changes in DOC concentration and/or water color during the years 1990-2009 in VK, a regime shift index (RSI) was calculated with a SRDS Software (www.climatelogic.com) according to Rodionov (2004), based on monthly mean values (May-September) of the variables measured at 1 m depth. The SRDS algorithm base on sequential Student t-tests for detecting time points of significant changes in the variable mean in a time series. For the RSI calculation we set the probability level at 0.05 and cut-off length as 50, corresponding to a 10-year period. A clear rise of DOC concentration took place around 2000 and 2001 (see Fig. 1), which was also detected by RSI. Thus, we compare VK data separately for the period of 'lower-DOC' years 1990-2000 and that of 'higher-DOC' years, 2001-2009 (later referred to as low-DOC_{period} and high-DOC_{period}, respectively). We also compare specifically data pooled for the three years of the lowest (1990, 1998, 2000, low-DOC_{yrs}) and the highest DOC concentration (2002, 2004, 2005, high-DOC_{yrs}). Sampling for most variables took place weekly or biweekly, thus n = 8-16 per ice-free season (i.e. June to September). Because normal distribution and data independence could not be ensured, non-parametric Mann-Whitney U test (SigmaPlot 13 software) was applied to test for statistical differences in the measured variables within the lakes between low-DOC_{period} and high-DOC_{period} as well as between low-DOC_{yrs} and high-DOC_{yrs}. In AM the pre DOC addition data from June-September 2007 were compared with those of the DOC addition years (2008 and 2009) and tested with Mann-Whitney U test, similarly to that done with VK data. During the years 2007 and 2008 most samples in AM were taken twice a month but in 2009 only once a month. Thus, the results of the both DOC addition years were pooled.

Although there were some differences in chemical properties (color, original DOC, total nitrogen, total phosphorus) between VK and AM (cf. Table 2), we estimated possible changes caused by cane sugar DOC addition on epilimnetic metabolism and phytoplankton community composition in AM by using VK as a 'reference lake' before (2007) and during

the DOC addition years (2008-2008). Because the number of measurements per month varied (1-4) and the sampling did not always take place during the same day, monthly mean values of the variables were compared with a paired t-test (SigmaPlot 13). In a case of failed normal distribution (4 of 24), non-parametric Wilcoxon Signed rank test was used. We compare basic results of water chemistry (samples from 1 m depth, except for dissolved organic carbon (DIC) from 0-0.30 m layer), carbon flux (phytoplankton primary production (PP, 0-1 m layer)), epilimnetic community respiration (CR, 0-1 m layer), and carbon dioxide (CO_2) efflux to the atmosphere collected during the summer stratification period (June – September). Phytoplankton biomass, based on microscopical counts, and crustacean zooplankton abundance in VK were from epilimnion (0-1 m), but in AM these were averaged for epi- and metalimnion (0-3 m). Thus, for these variables only within-lake differences between low-DOC and high-DOC periods and years were tested. Non-metric multivariate scaling (NMS) was used for visualizing differences in monthly phytoplankton community composition between different years (2007-2009) in VK and AM. The variables were plotted in the NMS figure as vectors. Differences in within-year variation of phytoplankton community composition were tested with the homogeneity of multivariate dispersion (PERMDISP)-routine in PERIMER 6 & PERMANOVA+ add-on (Anderson 2006).

Carbon dioxide (CO_2) efflux from both lakes was estimated with the boundary layer (BL) method (Cole and Caraco 1998, details in Kankaala et al. 2013) based on dissolved inorganic carbon (DIC) concentration and pH measured from the 0-30 cm top layer of the lakes and wind speed measurements at VK (Huotari et al. 2011). Although shown to underestimate CO_2 fluxes in VK (Huotari et al. 2011, Gålfalk et al. 2013, Erkkilä et al. 2018), the BL method was used due to a lack of high-frequency measurements of water temperature and meteorological drivers during the 1990s in VK as well as in AM studies, which are required for more accurate model estimations. The BL method does not take into account possible changes in

physical processes in the surface mixing layer related to DOC and color increase. Especially in the gas exchange of small lakes, the relative importance of convection may exceed that by wind mixing during summer stratification (Read et al. 2011). Although bulk of the CO_2 effluxes from these small headwater lakes usually takes place during autumn turnover period (Huotari et al. 2011), the data for October and November during the whole study period (1990-2009) were too sparse for reliable estimates for the whole open water period. However, the results should provide a reliable indication of the potential direction of changes related to DOC increase.

Perch growth was back-calculated from opercular bones in both lakes and the results are given here for three years (2005-2007) prior to DOC addition to AM, during the DOC addition years (2008-2009), and for years 2010 and 2011 after the manipulation. For details of methods, see Rask et al. (2014). Between-lake differences in the growth of young (1st and 2nd year) perch was tested with Mann-Whitney U test as well as for AM before (2007) and during (2008 and 2009) the sugar manipulation.

Results and Discussion

Long-term and short-term changes in VK

Water chemistry and carbon flux

The long-term increase in DOC concentration and water color (Fig. 1) in VK, from the 1990s to the next decade (from ca. 11 to 13 mg C L^{-1} and from 140 to 160 mg Pt L^{-1}, respectively), were strongly related to recovery from acidification of the lake and its catchment, indicated by a significant increase in alkalinity (Vuorenmaa et al. 2014, Lehtovaara et al. 2014, Table 1). Water pH varied more than alkalinity and showed only a minor increase presumably because of the impact of organic acids transported from the catchment by inflow (Arvola et al. 2010). The regime shift index (RSI, see methods) for DOC indicates that a clear sudden increase

took place around the years 2000-2001 (Fig. 1), but not simultaneously with that in color values (most pronounced shift in 2004). In this long-term dataset, color explained only 56% of the DOC variation by a linear regression equation: DOC (mg C L^{-1}) = 0.04 x color (mg Pt L^{-1}) – 6.05), (R^2 = 0.56, n = 100). Thus, as shown in many previous studies, browning is also influenced by other factors such as iron leaching from soils and its complexation with DOC (Krizberg and Ekström 2012, Weyhenmeyer et al. 2014) as well as differences in molecular structure and the proportion of colored substances in DOC, shown by spectral absorbance characteristics (e.g. Köhler et al. 2013, Adams et al. 2018).

In a three year study (2007-2009) at the riparian zone and in the lake, Rasilo et al. (2015) observed that DOC concentration in VK was positively correlated with catchment runoff, but negatively with weekly rainfall, air, soil, and lake-surface water temperatures. During the whole study period (1990-2009) the annual variation in DOC concentration in VK was not clearly related to that in mean precipitation and air temperature (cf. Suppl. Fig. 1). For example, of the three years with the highest annual precipitation (1998, 2004 and 2008) only 2004 was one of the high-DOC_{yrs} in our dataset. In whole ecosystem-scale carbon flux measurements during 15 years in two small boreal forest catchments, Pumpanen et al. (2014) showed that precipitation largely determined DOC fluxes, but also the forest (ca. 50 year-old Scots pine, *Pinus sylvestris* stand) net ecosystem exchange and litter production during the previous year had a small but significant effect on DOC runoff. Thus, presumably also time lags in DOC leaching from the soil and reaching the lake affected the DOC concentration variation in the headwater lake VK.

The concentration of DIC in the lake surface layer and CO_2 efflux to atmosphere as well as community respiration (CR) in the epilimnion were significantly greater (Mann Whiney U test, p = 0.021, 0.029, 0.024, respectively, Table 1, Suppl. table 1) and oxygen concentration lower ($p < 0.001$) during the high-DOC_{period} compared with those during the low-DOC_{period}.

However, when only the measurements from low-DOC_{yrs} (1990, 1998, 2000) and high-DOC_{yrs} (2002, 2004, 2005) were compared, no significant differences were detected in those, except for oxygen concentration ($p < 0.001$, Suppl. table 1), suggesting that inter-annual variation in CO_2 efflux may not be directly coupled with DOC and CR. The bulk of annual CO_2 release takes place during erosion of stratification and water column mixing in late summer and autumn (Huotari et al. 2011), which were not fully covered in our dataset of biweekly samples from June-September. Moreover, CO_2 originating from catchment and riparian soil respiration also contributes to CO_2 concentrations and fluxes from headwater streams and lakes (Rasilo et al. 2012, Dinsmore et al. 2013), uncoupled from DOC decomposition in the lake itself.

No significant changes in total nitrogen (TotN) and phosphorus (TotP) concentration took place during the study period in VK (Mann Whitney U test, $p > 0.05$, Table 1), although the concentration of dissolved inorganic nitrogen (DIN, i.e. NO_3-N, NO_2-N and NH_4-N) slightly increased (Vuorenmaa et al. 2014). This was presumably more related to in-lake processes, like changes in phytoplankton nutrient uptake in the light-limited environment, and to hydrology (Vuorenmaa et al. 2014, Arvola et al. 2014), than those taking place in the catchment; N deposition declined (Ruoho-Airola et al. 2014) and net retention of inorganic N in the catchment was high (Forsius et al. 2005). However, the TotN concentrations were significantly higher during high-DOC_{yrs} compared with those during the low-DOC_{yrs} ($p < 0.001$, Suppl. table 1). Thus, although not clearly seen in VK long-term data, totN input seemed to be connected with DOC during the high-DOC_{yrs}. In VK the relationship between N and DOC as well as between lake DIN and atmospheric N deposition differed from those observed on broad regional and/or time scale surveys in the same climatic and vegetation zone. In data collected from of unmanaged boreal forest catchments from southern to northern Finland (n=21) during several years, annual median and/or mean export estimates for total

organic carbon and N were strongly correlated and totN consisted in average 85% of organic N (Kortelainen et al. 2006). However, in the long-term parallel measurements in VK, DOC and organic N concentrations were not correlated (Vuorenmaa et al. 2014). In a large dataset, covering 78 headwater streams and 95 nutrient-poor lakes in Sweden sampled during the period 1998-2013, average epilimnetic (July-September) DIN concentrations decreased and DIN:totP ratios declined in a strong correlation with diminishing atmospheric N deposition (Isles et al. 2018). This phenomenon was not seen in VK during the study period 1990-2009, for which no obvious explanation exists. Presumably, several counteracting, non-synchronous and catchment-specific factors, like variable organic matter loading, decreased N deposition and changes in-lake processes influenced N processing in the old-forest catchment and the lake.

Along with browning and DOC increase, a long-term decrease in primary production (PP) was evident in VK (Arvola et al. 2014, Peltomaa 2013), as also seen in the data grouped for low-DOC and high-DOC periods and years (Table 1, Suppl. table 1). Seekell et al. (2015) detected nonlinearity in the response of PP to allochthonous DOC in small arctic and boreal lakes. Below DOC concentration of ca. 5 mg C L^{-1}, primary producers benefit from additional N and P transported with allochthonous organic matter, but at higher DOC concentrations the effect is negative. In VK the DOC concentration was already at the beginning of the monitoring period >8 mg C L^{-1}, thus, supporting the conclusion drawn by Seekell et al. (2015). However, the long-term change in VK was not clearly detected in short-term measurements. In weekly measurements during three years (2002-2004) Peltomaa and Ojala (2016) observed no clear immediate response in pelagic metabolism (PP, CR and ^{14}C-leucine uptake) after sudden rain events. After a long rainy period with some extreme rain peaks (20-50 mm d^{-1}) in 2004, water color in the epilimnion increased markedly but no clear immediate increase was observed in DOC concentration. The photic layer of the lake become shallower,

which caused a decrease in PP at 1 m depth but did not affect PP in the uppermost 0.5-m layer where most PP occurred.

Plankton community

Total phytoplankton biomass, based on microscopical counts, was significantly lower in VK during the high-DOC years and periods (Mann Whitney U test, p = 0.002, <0.001, respectively, Table 1, Suppl. table 1) although the differences based on chlorophyll *a* were significant only when low-DOC_{yrs} and high-DOC_{yrs} were compared (p = 0.013, Suppl. table 1). Changes in phytoplankton community composition also took place: the biomass of Chlorophytes declined whereas that of Cryptophytes and Chrysophytes slightly increased during the high-DOC_{period}. Many taxa among Cryptophytes and Chrysophytes are potentially mixotrophic (e.g. Jones 2000), and thus, presumably benefitted from high-DOC conditions and associated bacteria. Flagellate taxa, capable of diurnal vertical migration for nutrient uptake from dark, nutrient-rich hypolimnion and photosynthesis in the euphotic zone, are typical in humic lakes (Salonen et al. 1984, Lepistö and Rosenström 1998, Drakare et al. (2002) observed that the proportion of autotrophic and mixotrophic flagellates in phytoplankton increased along a DOC gradient from 3 to 31 mg C L^{-1} in small lakes in northern Sweden.

In VK, the raphidophyte *Gonyostomum semen* formed ca. 40 % of the total phytoplankton biomass, while its annual and seasonal contribution varied a lot. For the period 1990-2003 Peltomaa et al. (2013) detected a slight decline of *G. semen* biomass, along with totP concentration, but in the data for the whole study period (1990-2009) no clear trend can be seen. Annual median biomass variation of this taxon was not related to DOC concentration. The species is typically abundant in humic lakes (Gronberg et al.1988, Angeler et al. 2012). In monitoring data collected from the epilimnion of 95 Swedish lakes in August over 5 years,

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blooms of *G. semen* were typical in acidic, brown-water lakes with totP concentration >15 µg L^{-1} (Lebret et al. 2018). However, the blooms were more associated with high iron concentration (ca. 200 µg L^{-1}) than with total organic carbon contributing to lake browning (Lebret et al. 2018). In VK, pH and totP (cf. tables 1 and 2) as well as epilimnetic iron concentrations (average±SD 225±43 µg L^{-1}, measured at 1 m depth in June –August 1994-2005 and 2009, Finnish Environment Institute, open data, https://www.syke.fi/avointieto) were very similar to those in Swedish lakes with *G. semen* blooms. On the other hand, this vertically migrating species also efficiently utilizes N and P resources from the anoxic hypolimnion in VK (Salonen and Rosenberg 2000), where the concentration of iron is also higher (677±128 µg L^{-1} at 5-6 m depth, the period and data source as above) than in the epilimnion.

During the study period 1990-2009, changes in crustacean zooplankton species dominance were recorded both in cladocerans and copepods. *Ceriodaphnia quadrangula* was the dominant cladoceran in most years in the 1990s but was gradually replaced by *Bosmina longirostris* during the years of regime shift (2002-2004) for DOC and water color. Among copepods, *Mesocyclops leuckarti* was most abundant in the early 1990s but subsequently *Thermocyclops oithonoides* dominated (Lehtovaara et al. 2014). The changes in zooplankton community were associated with increasing carbon load and recovery from acidification but changes in the microbial community and phytoplankton, including the decrease in primary production (Arvola et al. 2014), may also have had an influence. Within the food web, bottom up regulation of zooplankton seemed to exceed the importance of top-down control (Lehtovaara et al. 2014). However, there were no significant differences in the total abundance of cladocerans and copepods between high-DOC and low-DOC periods and/or years ($p>0.05$, Table 1, Suppl. table 1). This differs from the results obtained for two small lakes in northeastern Pennsylvania, USA, undergoing recovery from acidification and

browning during a 27-year monitoring period; the abundance of *Daphnia* and calanoids decreased, whereas the abundance of cyclopoids increased or was unchanged (Williamson et al. 2015). The DOC concentration in these lakes was originally much lower than in VK and increased ca. 1 mg C L^{-1} (from ca. 1 to 2 and 5 to 6 mg C L^{-1} , respectively) during the monitoring period.

Fish

In VK, long-term changes took place in the population dynamics of perch, as the occurrence of strong year-classes every four years in the 1990s levelled off in the 2000s (Rask et al. 2014, Table 1). Around 2000-2005, i.e. the years of the regime shift (see above), also a significant decrease was recorded in perch growth in the first two years of life. This was related to the increase in water color and subsequent decrease of the general productivity of the lake, decrease in volume of oxygenated habitat and deterioration of light conditions for perch, which a visual feeder (Rask et al. 2014). The slight increase in the growth of older and larger perch in the same years suggested their lower dependence on the pelagic food web and feeding on macroinvertebrates (Rask et al. 2014).

Short-term effects in AM in comparison to VK

Water chemistry and carbon flux

During the study of AM (2007-2009), the reference lake VK was already in the 'high-DOC' phase. Rasilo et al. (2015) estimated that during these years the annual load of terrestrial DOC to VK varied between 1500 and 4100 kg, which means ca. 37-100 g C m^{-2} yr^{-1} per lake surface area. Assuming that terrestrial DOC input to AM was of the same order of magnitude as that to VK, the cane sugar addition in 2008 and 2009 was ca. 18-37% of the annual DOC input to the lake. However, when the labile low-molecular weight fraction is generally <20% terrestrial DOC (Münster 1993, Jonsson et al. 2007, Kiikkilä et al. 2011), the cane sugar

addition most likely exceeded the input of labile DOC transported from the surrounding catchment; the DOC concentration increased by 2 mg C L^{-1}, thus, reaching the level prevailing in VK during those years (Table 2).

Because monthly mean temperatures, measured at 1 m depth in both lakes, did not differ significantly (paired t-test, $p > 0.05$, Table 2), we assumed that seasonal weather-induced variations in both lakes were similar and, thus, the paired mean monthly data could be used for the comparisons. During the pre DOC add year 2007, CO_2 efflux, PP and CR and water pH did not differ significantly ($p > 0.05$) between the lakes even though DOC, totP, totN and chlorophyll *a* concentrations as well as color values were significantly higher in VK, ($p < 0.05$ in all, Table 2). Cane sugar caused no change in water color, as expected, and no marked changes in PP took place during the study years (Peura et al. 2014, Table 2, suppl. Table 2), as expected.

In AM, significantly greater surface DIC concentrations and CO_2 effluxes were measured during the DOC addition years (paired t-test, $p<0.001$ in both), suggesting immediate increase in DOC decomposition. However, this was not clearly seen in the measurements of epilimnetic community respiration (CR), bacterial production and O_2 concentration (Suppl. table 2, Peura et al. 2014). The measurements were done two weeks after DOC amendments and presumably some microbial activity peaks were missed in the measurements. However, a slight but significant decrease in pH and alkalinity in AM during the DOC addition years were presumably related to increased concentration of CO_2 (Mann Whitney U test, $p < 0.001$, 0.017, Suppl table 2, see also Table 2), thus, in the opposite direction from those taken place in VK.

Plankton community

Phytoplankton community composition in VK and AM clearly differed. In June-September 2007-2009, 58-81% of the biomass in VK consisted of the raphidophyte *G. semen* (in average 67%) and only in one case was the proportion of chrysophytes higher (69%) than that of *G. semen*. Cryptophytes, dinophytes, chrysophytes, diatomophytes and chlorophytes averaged 4, 9, 15, 2 and 1%, respectively, of the biomass. In AM, before cane sugar addition in 2007, chrysophytes and chlorophytes averaged 60% and 39%, respectively, of the phytoplankton biomass. The proportion of cryptophytes, dinophytes and *G. semen* was then ca. 1-2% of the biomass. During the DOC addition years the total phytoplankton biomass was at the same level as in 2007 (Suppl. table 2). However, changes in the community composition occurred; the proportion of chrysophytes declined and that of *G. semen* increased. In the latter half of the second DOC manipulation season (August-September 2009) up to 37-75% of the phytoplankton biomass consisted of this taxon. Thus, although no water browning took place, *G. semen* was favored by labile DOC increase and presumably also by the lowered pH (see Lebret et al. 2018, and discussion earlier). The biomass of cryptophytes was slightly, but significantly higher during the DOC addition years compared with that in 2007 (Mann Whitney U test, $p = 0.028$, Suppl. table 2). Also the proportion of heterotrophic taxa (e.g. *Bicosoeca* spp., *Petalomonas* sp., *Katablepharis ovalis*) increased from ca. 1% in 2007 to 4% during the DOC addition years. PERMDISP analysis indicated that the seasonal variation in phytoplankton community composition increased in AM during the DOC addition years ($F_{2,9} = 9.069$, $p = 0.02$, Fig. 2) while the variation in VK did not change ($F_{2,9} = 2.981$, $p = 0.299$). Hence, it seems that the phytoplankton community became more stochastic in AM following the DOC additions.

Zooplankton community composition also differed between VK and AM. In VK copepods were more abundant than cladocerans (Table 2). In AM the crustacean zooplankton community was dominated by the cladocerans *Holopedium gibberum, C. quadrangula,* and

Daphnia sp. and by small cyclopoids. Enriched stable carbon isotope values ($\delta^{13}C$) of particulate organic matter and zooplankton clearly showed transfer of cane-sugar DOC to zooplankton during the DOC addition years (Peura et al. 2014), although no significant differences were observed in the abundance of cladocerans and copepods during the study years (Mann Whiney U test, $p > 0.05$, Suppl. Table 2). This differs from the result obtained by Kelly et al. (2016) in a DOC manipulation experiment of an oligotrophic temperate lake, where the catchment-origin DOC concentration in the treatment basin increased from 8 to 11 mg C L^{-1} whereas in the reference basin DOC concentration decreased from ca. 8 to 6 mg C L^{-1}. The authors concluded that zooplankton in the treatment basin benefitted from improved resource quality due to an increase in PP associated with transported phosphorus (see also Seekell et al. (2015) for the positive impact on PP, but Williamson et al. (2015) for negative long-term changes in zooplankton in the lakes with DOC concentration <6 mg C L^{-1}). In our study lakes DOC concentration was originally higher (ca. 10-11 mg C L^{-1}) and the long-term effect on PP was negative in VK and the short-term effect insignificant in both lakes, resulting no clear effect on crustacean zooplankton density.

Fish

During the DOC addition experiment in AM (2007-2009), the density and biomass of the perch population in both lakes (AM and VK) were similar (600-1500 and 1000-1700 ind/ha, and 14-28 and 22-27 kg/ha.).

The first year median growth of perch was higher in AM than in VK during 2005-2010 ($U=36$, $p=0.004$). In VK both the 1st and 2nd year growth of perch showed less variation than in AM. The second year growth of perch was similar in both lakes during 2005-2007 but in the years of DOC manipulation (2008 and 2009) the growth was significantly faster in AM (Mann-Whitney $U=408$ and 352, $p=0.04$ and <0.001, respectively, Fig. 3). When only the

"reference year" 2007 and DOC "manipulation years" 2008 and 2009 were considered, the first year growth of perch in AM was significantly higher in the reference year (Mann-Whitney U=561, p=0.005) but the second year growth during the manipulation years was greater (Mann-Whitney U=721, p<0.001). Enriched stable carbon isotope values ($\delta^{13}C$) revealed the importance of the route of benthic invertebrates to fish leading to a cane sugar carbon proportion of 18.7 % of perch carbon biomass at the end of the sugar addition (Jones et al. 2018). The extra cane-sugar DOC input seemed to temporarily stimulate more the growth of benthivorous 2nd year perch than that of primarily planktivorous young-of-the-year perch. The diet of perch was not examined in this study, but the increasing proportion of benthic food during the early years of perch has been recorded in other studies and lakes in the study area (Rask and Arvola 1985, Estlander et al. 2010). The slight increase in the growth of older and larger perch in VK (Rask et al. 2014) suggests that increasing DOC may positively affect benthivorous fish also in natural conditions.

Terrestrial DOC vs. cane sugar DOC and long-term monitoring vs. short-term manipulation – What can be learned?

The extensive dataset collected during long-term monitoring shows clear ecosystem-scale changes and a regime shift due to DOC increase and browning in VK: poorer light penetration into the water column was followed by changes in community structure, decrease in PP and in the growth of young perch, as well as increase in CO_2 efflux to the atmosphere. In the short-term (2 years) manipulation with cane sugar in AM, the pathway of DOC up to fish could be traced by $\delta^{13}C$ and also an increase in CO_2 efflux could be observed. However, detecting significant changes in a strict statistical sense was limited by marked seasonal and inter-annual variation in the measured variables and rather low frequency of measurements. For example, epilimnetic bacterial cell number and production as well as CR were not clearly related to DOC addition to AM (Peura et al. 2014). However, in short (2-3 weeks) replicated

mesocosm experiments significant increase in these variables indicating heterotrophy is generally seen after DOC addition (Kankaala et al. 2010, Faithfull et al. 2012).

Glucose or sucrose, as here in the form of cane sugar, are commonly used to simulate labile DOC in experiments (e.g. Blomquist et al. 2000, Smith and Prairie 2004, Faithfull et al. 2012), and could represent fresh biodegradable detritus of both autochthonous and allochthonous origin (cf. Søndergaard et al. 2000, Kiikkilä et al. 2011). In addition to carbohydrates (including sugars), low-molecular-weight terrestrial DOC contain other compounds like amino acids and carboxylic acids, which also are easily degraded by microbes (Jonsson et al. 2007, Berggren et al. 2010, Kiikkilä et al. 2011). However, more than half of terrestrial DOC consists of high-molecular-weight humic compounds, which are recalcitrant to immediate degradation by aquatic microbes (e.g. Jonsson et al. 2007, Räsänen et al. 2018). Thus, the impacts of colored humic substances are rather negative than positive to lake food webs by reducing light availability for primary producers (Thrane et al. 2013, Kelly et al. 2018) and thus, impairing food resources available for the higher trophic levels (Karlsson et al. 2015, Taipale et al. 2016). Moreover, colored substances promote steeper temperature and oxygen stratification in summer, which narrow down potential habitats for fish survival and worsen foraging opportunities for visually feeding fish (Estlander et al. 2010, Williamson et al. 2015).

Although DOC enhances bacterial production, growth efficiency of bacteria utilizing both low-molecular-weight and high-molecular-weight DOC is strongly related to availability of inorganic nutrients (N, P) (Smith and Prairie 2004, Jansson et al. 2006, Räsänen et al. 2018), which also impacts on the magnitude of DOC-origin carbon available for consumers and/or respired and finally released as CO_2 to the atmosphere. Although available for grazers, bacteria are poor-quality diets for zooplankton and zoobenthos due to lack of essential polyunsaturated fatty acids and sterols (Brett and Müller-Navarra 1997, Martin-Creuzburg et

al. 2011, Taipale et al. 2012). Even when the experimental addition of labile DOC to AM (22 g m^{-2} yr^{-1}) was of the same order of magnitude as annual PP (AM 18-20 g C m^{-2} yr^{-1}, Peura et al. 2014), only <20% of top consumer (perch) carbon originated from labile DOC (Jones et al. 2018). This indicates the inefficiency of DOC-based food chains and consumer requirement for their diets to include high-quality food resources produced by algae (Taipale et al. 2016).

Although the $\delta^{13}C$ label of DOC addition could be detected in AM consumers (crustacean zooplankton, benthic invertebrates, fish) this was not the case for the components of seston <100 µm (phytoplankton, bacteria, detritus, protozoans, rotifers), which could not be separated and analyzed, so that the actual microbial food chain routes could not be followed. In addition to pelagic and benthic microbial food chains, flocculation of cane-sugar DOC to particulate organic carbon (POC) (see von Wachenfeldt and Tranvik 2008) could have been another route for benthic consumers, contributing to their $\delta^{13}C$ enrichment. Although the flocculation of cane sugar in AM is speculative, the results from a whole-lake scale experiment with maize leaf addition into two German lakes showed that benthic invertebrates could play a significant role in transferring terrestrial POC to fish (Scharnweber et al. 2014). However, the contribution of maize leaves, corresponding to the autumnal alder leave input, was only 1-3% of omnivorous fish carbon and the benthic microbial food chain in these lakes proved to be inefficient (Lischke et al. 2017).

In conclusion, long-term monitoring of a boreal headwater lake confirmed the general trend of increasing DOC concentration and browning in northern lakes and associated changes in pelagic community structure as well as in productivity. The decrease in lake primary production was also reflected in higher trophic levels, here the growth of young perch. Both long-term monitoring and whole-lake DOC manipulation showed increase in CO_2 efflux to the atmosphere and changes in pelagic community structure followed by the increase in DOC concentration. Long-term integrated monitoring of aquatic ecosystems is without question

important to reveal responses of lake parameters to environmental change. However, given normal high between-lake variability, data obtained from an individual lake and catchment will not necessarily support trends based on correlation or regression analyses of parameters measured on large regional and/or time scales. The whole-lake manipulation approach has an advantage of detecting ecosystem-scale responses on one major driver, as here on labile DOC, but not simultaneously affecting other factors like browning also suppressing light availability for primary producers. This experimental manipulation, applying $\delta^{13}C$ differences in cane-sugar and catchment-origin DOC, revealed the importance of benthic pathways from DOC to perch (Jones et al. 2018). This was not possible to detect in the long-term monitoring of VK and in the mesocosm experiments, although both these approaches detected increased pelagic heterotrophy after DOC increase (cf. Table 1, Kankaala et al. 2010). Short-term whole-lake manipulation experiments can provide insights into the processes behind the patterns and trends demonstrated in long-term studies.

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Table 1. Median values (with 25-75% range) of water chemical variables at 1 m depth (except DIC at 0-30 cm), CO_2 efflux from lake surface, phytoplankton primary production (PP) and community respiration (CR, uppermost 1 m layer), phytoplankton biomass (PB), and zooplankton density in June – September (epilimnion) during the 'low-DOC_{period}' (1990-2000) and 'high-DOC_{period}' (2001-2009) as well as those of perch density and growth during the first and second year in Lake Valkea-Kotinen during the same periods. Statistical differences between the periods was tested with Mann Whitney U test (p denote significance, ns = not significant).

	Low-DOC_{period} (1990-2000)			High-DOC_{period} (2001-2009)			
Variable	median	range (25-75%)	n	median	range (25-75%)	n	p
DOC mg C L^{-1}	11.3	(10.3 - 12.1)	207	12.9	(12.4 - 13.6)	170	**<0.001**
color mg Pt L^{-1}	135	(124 – 147)	192	162	(152 – 179)	170	**<0.001**
pH	5.2	(5.1 - 5.4)	192	5.3	(5.1 - 5.4)	162	**0.025**
Alkalinity mmol L^{-1}	0.005	(0.0009 - 0.0175)	177	0.087	(0.013 - 0.023)	146	**0.006**
O_2 mg C L^{-1}	8.9	(8.4 - 9.5)	190	8.1	(7.4 - 8.8)	219	**<0.001**
TotN µg L^{-1}	460	(412 – 526)	298	461	(436 – 504)	242	ns
TotP µg L^{-1}	15	(13 – 18)	298	15	(13 – 18)	242	ns
DIC mg C L^{-1}	0.40	(0.27 - 0.61)	192	0.48	(0.30 - 0.78)	146	**0.021**
CO_2 eff. mg C m^{-2} d^{-1}	138	(58 – 288)	192	201	(74 – 393)	146	**0.029**
PP mg C m^{-2} d^{-1}	116	(82 - 149)	190	75	(52 – 103)	132	**<0.001**
CR mg C m^{-2} d^{-1}	100	(74 - 131)	190	115	(81 – 149)	132	**0.024**
Chlorophyll*a* µg L^{-1}	13.0	(9.19 - 21.2)	190	13.7	(10.7 - 16.8)	132	ns
PB Tot mm^3 L^{-1}	2.15	(1.29 - 3.54)	220	1.70	(1.07 - 2.60)	171	**0.002**
Cryptophytes mm^3 L^{-1}	0.05	(0.02 - 0.11)	220	0.06	(0.03 - 0.11)	171	**0.019**
Dinophytes mm^3 L^{-1}	0.05	(0.01 - 0.21)	220	0.08	(0.02 - 0.25)	171	ns
Chrysophytes mm^3 L^{-1}	0.09	(0.02 - 0.23)	220	0.21	(0.11 - 0.35)	171	**<0.001**
Diatomophytes mm^3 L^{-1}	0.02	(0.01 - 0.05)	220	0.02	(0.01 - 0.07)	171	ns
Raphidophytes mm^3 L^{-1}	0.68	(0.11 - 1.74)	220	0.73	(0.07 - 1.61)	171	ns
Chlorophytes mm^3 L^{-1}	0.08	(0.02 - 0.19)	220	0.03	(0.02 - 0.06)	171	**0.001**
Cladocera, ind. L^{-1}	2.1	(0.2 - 15.5)	190	3.5	(0.7 - 12.5)	113	ns
Copepoda, ind. L^{-1}	26.6	(11.3 - 57.5)	190	30.0	(15.5 - 67.4)	113	ns
Perch density, ind ha^{-1}	1107	(738 – 1738)	10	1214	(988 – 1298)	9	ns
Perch, 1st year growth, mm	62.0	(59.3 – 64.0)	995	50.8	(49.5 - 56.8)	459	**<0.001**
Perch, 2nd year growth, mm	38.1	(31.3 - 43.4)	967	31.8	(30.6 - 32.8)	405	**<0.001**

Table 2. Mean (±SD) monthly values of June-September temperature and those of chemical variables (1 m depth, except DIC from 0-0.3 m depth) as well as carbon flux (CO_2 efflux from lake surface and primary production (PP) and community respiration (CR) in the 0-1 m layer) in VK and AM before (2007) and during the cane sugar addition to AM (2008-2009). The differences were tested with paired t-test of monthly mean values
805 based on 1-4 measurements per month (for t-test n=4 in 2007, 8 in 2008-2009 in both lakes). The results for the total phytoplankton biomass and cladoceran and copepod abundances in VK are only for epilimnion, but in AM averged for epi- and metalimnion, and, thus, statistical differences were not tested. Differences in the 1st and 2nd year growth of perch was tested with Mann-Whitney U test (n = 21 and 42 (VK) and 44 and 66 (AM) for 1 and 2 yr perch in 2007 and 16 and 58 (VK) and 17 and 39 (AM) for 1 and 2 yr perch in 2008-2009).

Paired t-test in SigmaPlot 13	2007 pre DOC add year June-Sep					2008-2009 during DOC add years June-Sep				
	VK		AM			VK		AM		
Variable	mean	SD	mean	SD	p	mean	SD	mean	SD	p
Temperature ˚C	16.7	2.5	15.6	3.5	0.136	15.6	2.6	15.7	2.3	0.960
DOC mg C L^{-1}	12.2	0.5	10.4	0.4	**0.008**	12.4	0.3	12.0	0.8	0.256
color mg Pt L^{-1}	162	7	98	10	**<0.001**	167	8	99	4	**<0.001**
pH	5.2	0.1	5.1	0.0	0.337	5.2	0.254	4.9	0.1	**0.008**
Alkalinity mmol L^{-1}	0.006	0.004	-0.012	0.003	**0.010**	0.005	0.002	-0.019	0.007	**0.008**
O_2 mg C L^{-1}	8.3	1.1	8.3	0.5	0.949	8.4	0.5	7.9	0.7	*0.085*
TotN µg L^{-1}	515	77	378	8	**0.036**	462	21	377	26	**<0.001**
TotP µg L^{-1}	20	5	10	2	**0.026**	20	2	12	2	**<0.001**
DIC mg C L^{-1}	0.683	0.403	0.693	0.370	0.845	0.613	0.360	0.925	0.380	**<0.001**
Chlorophyll*a* µg L^{-1}	15.9	6.0	5.1	0.6	**0.037**	15.0	3.1	6.6	5.5	**0.016**
CO_2 eff. mg C m^{-2} d^{-1}	267	202	268	143	0.979	240	190	484	196	**<0.001**
PP mg C m^{-3} d^{-1}	78	24	83	26	0.541	66	19	81	54	0.351
CR mg C m^{-3} d^{-1}	127	75	78	28	0.427	99	37	80	23	0.202
PhytoB Tot.mm^3 L^{-1}	2.4	0.7	1.0	0.7		2.7	0.6	1.1	0.6	

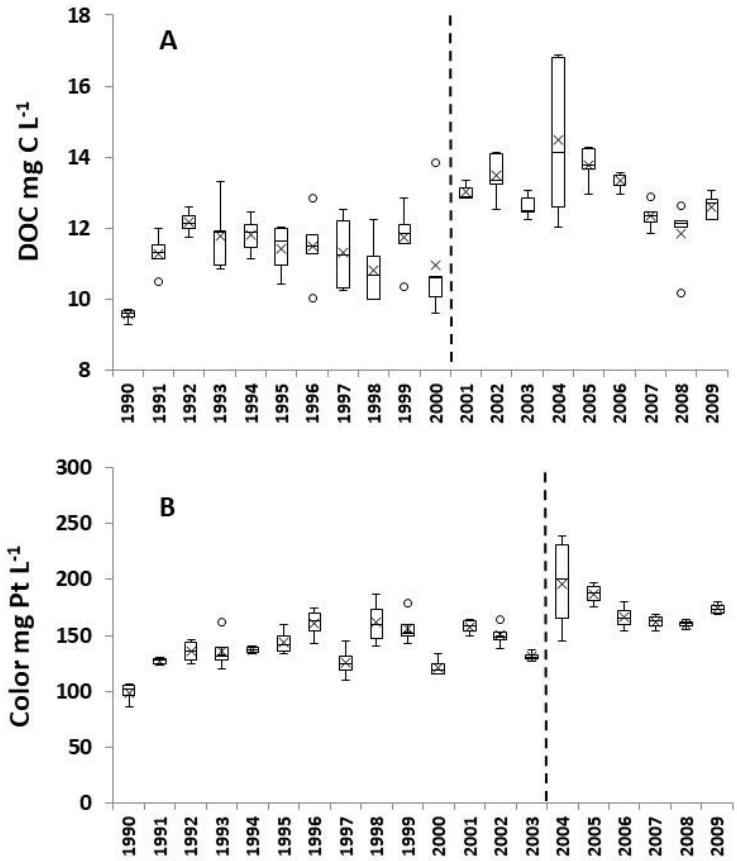
Cladocera, ind. L^{-1}	6.6	3.3	18.4	13.7		8.5	7.4	19.5	16.5	
Copepoda, ind. L^{-1}	66.3	47.1	8.3	9.0		69.2	53.7	16.9	16.6	
Perch density, ind ha^{-1}	775.0		n.d.			1238	352.0	1040.0	646.0	1.000
Perch, 1st year growth, mm	49.8	5.2	69.1	6.4	**<0.001**	52.4	4.6	63.7	6.9	**<0.001**
Perch, 2nd year growth, mm	31.1	4.1	31.1	11.3	0.767	31.7	5.7	34.4	8.1	**<0.001**

Figure legends

Fig. 1. A) Median DOC concentration (mg C L^{-1} with 25 and 75% quartiles and outliers, x indicates mean value) in Valkea-Kotinen during the years 1990-2009 B) Median and mean water color (mg Pt L^{-1}, symbols as in A) in the lake during the same period. Dash lines show regime shift time points calculated as RSI (DOC = 0.87, color = 0.93 $p < 0.001$ in both) according to Rodionov (2004), see methods for details.

Fig. 2. NMS figure visualizing the changes in phytoplankton community composition (group proportions) in Valkea-Kotinen (VK, reference lake) and Alinen Mustajärvi (AM) during the pre-manipulation (2007) and manipulation years (2008-2009). Markers represent monthly mean values, and the samples within a given year have been connected with a line. Variables are presented as vectors. The final stress for the 2-D solution was 0.05.

Fig. 3. First year (A) and second year growth (B) of perch in lakes Alinen Mustajärvi (white) and Valkea-Kotinen (grey) given as annual length increments for the years 2005-2011. Box plots indicate median values, first and third quartiles, and range. Horizontal arrow indicates the seasons of cane sugar addition.


A
DOC mg C L-1
18
16
14
12
10
8
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
B
Color mg Pt L-1
300
250
200
150
100
50
0

830

Fig.1

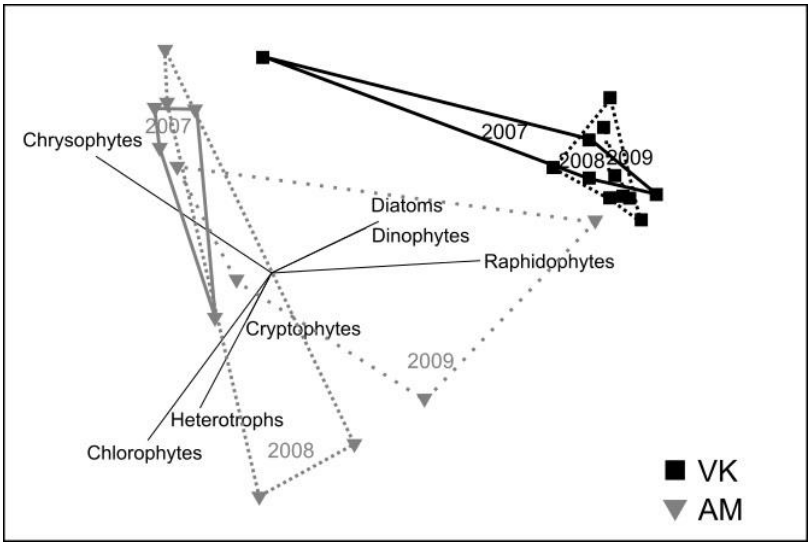

Chrysophytes
2007
2007
2008
2009
Diatoms
Dinophytes
Raphidophytes
Cryptophytes
2009
Heterotrophs
Chlorophytes
2008
VK
AM

Fig. 2.

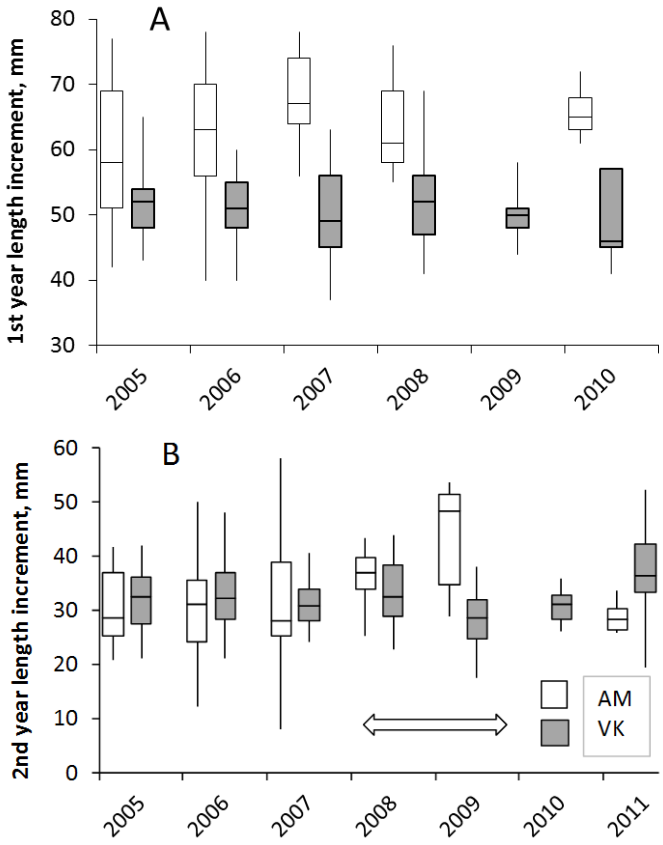

A
1st year length increment, mm
80
70
60
50
40
30
2005
2006
2007
2008
2009
2010
B
2nd year length increment, mm
60
50
40
30
20
10
0
2005
2006
2007
2008
2009
2010
2011
AM
VK

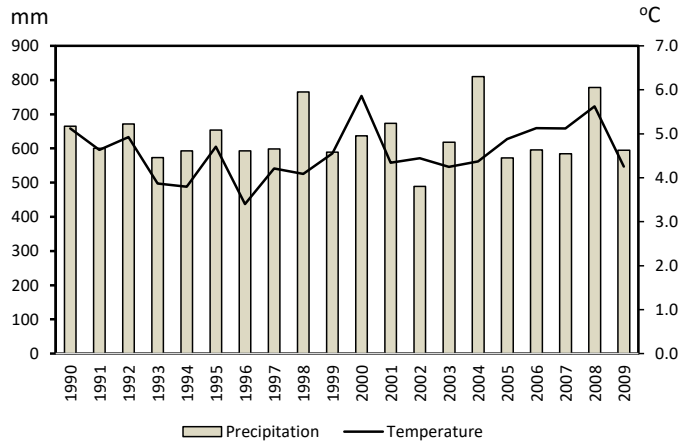
Fig. 3.

Suppl. Table 1. Results of Mann-Whitney U test comparing June-September data of low-DOC_{period} (1990-2000) vs. high-DOC_{period} (2001-2009) and those collected during low-DOC_{yrs} (the years 1990, 1998, 2000) vs. high-DOC_{yrs} (the years 2002, 2004, 2005) from Lake Valkea-Kotinen. Respective values comparing data collected from Lake Alinen Mustajärvi pre DOC (cane sugar) addition (2007) vs. those from DOC addition years (2008, 2009). (n separated with ; shows the number of measurements in respective calculations).

	Valkea-Kotinen Low-DOC_{period} vs. High-DOC_{period}				Valkea-Kotinen Low-DOC_{yrs} vs. High-DOC_{yrs}				Alinen Mustajärvi pre-DOC vs. DOC-add			
Mann-Whitney	U	p	n	response	U	p	n	response	U	p	n	response
DOC mg L^{-1}	3931	**<0.001**	198;134	+	118	**<0.001**	52;53	+	0	**<0.001**	9;13	+
color mg Pt L^{-1}	5400	**<0.001**	198;134	+	258	**<0.001**	52;53	+	47	0.457	9;13	
pH	13405	**0.025**	198;134	+	717	**<0.001**	52;53	+	1	**<0.001**	9;13	-
Alkalinity mmol L-1	458	**0.006**	198;134	+	10	**<0.001**	52;53	+	22.5	**0.017**	9:13	-
O_2 mg L^{-1}	11479	**<0.001**	198;134	-	1121	**<0.001**	52;53	-	57	0.412	8;16	
TotN µg L^{-1}	34738	0.515	198;134		859	**<0.001**	52;53	+	34	0.149	9;13	
TotP µg L^{-1}	35307	0.737	198;134		1285	0.362	52;53		50	0.776	9;13	
DIC mg L^{-1}(0-0.3 m)	14398	**0.021**	198;134	+	1592	0.856	52;53		28	**0.045**	9;13	+
CO_2 efflux mg C m^{-2} d^{-1}	12076	**0.029**	198;134	+	1336	0.790	52;53		27	**0.038**	9;13	+
Prim. prod. mg C m^{-2} d^{-1}	5895	**<0.001**	198;134	-	466	**<0.001**	48;46	-	32	0.158	9;13	
Comm. resp. mg C m^{-2} d^{-1}	7762	**0.024**	198;134	+	868	0.983	48;46		29	0.405	8;14	
Chlorophyll*a* µg L^{-1}	14794	0.471	198;134		1033	**0.013**	48;46	-	45	0.967	8;10	
Totphyto. biom. mm^3L^{-1}	15431	**0.002**	198;134	-	1083	**<0.001**	54;54	-	32	1	8;8	
Cryptophytes	16212	**0.019**	198;134	+	1607	0.812	54;54		12	**0.028**	8;8	+
Dinophytes	17075	0.168	198;134		1332	0.075	54;54		23	0.382	8;8	
Chrysophytes	11838	**<0.001**	198;134	+	1546	0.562	54;54		19	0.195	8;8	
Diatomophytes	17781	0.523	198;134		1128	**0.003**	54;54	-	32	0.959	8;8	
Raphidophytes	17635	0.385	198;134		1415	0.189	54;54		18	0.161	8;8	
Chlorophytes	12209	**<0.001**	198;134	-	543	**<0.001**	54;54	-	30	0.878	8;8	
Cladocera ind L^{-1}	9812	0.210	99;63		352	0.105	37;37		33	0.095	9;13	
Copepoda ind L^{-1}	9672	0.150	99;63		458	0.925	37;37		50	0.593	9;13	
Perch density, ind. ha^{-1}	U=38	0.568	11;9									
Perch, 1st year growth, mm	U=85	**<0.001**	995;459						U=561	**0.005**	44;17	-
Perch, 2nd year growth, mm	U=80	**0.020**	967;405						U=721	**0.000**	66;39	+

Suppl. Table 2. Median values (with 25-75% range) of water chemical variables at 1 m depth (except DIC at 0-30 cm), CO_2 efflux from lake surface, phytoplankton primary production (PP) and community respiration (CR, uppermost 1 m layer), phytoplankton biomass (PB, epi- and metalimnion), and zooplankton density (epi- and metalimnion) in June – September before DOC (cane sugar) addition (Pre-DOC, 2007) and during the DOC addition years (DOC-add, 2008-2009) as well as those of perch density and growth during the first and second year in Lake Alinen Mustajärvi during the same periods. Statistical differences between the periods was tested with Mann Whitney U test (p denote significance, ns = not significant).

	Pre-DOC (2007)			DOC-add (2008-2009)			
Variable	median	range (25-75%)	n	median	range (25-75%)	n	p
DOC mg C L^{-1}	10.0	(9.5 - 10.3)	8	12.0	(11.3 - 12.6)	8	**<0.001**
color mg Pt L^{-1}	97	(90 – 105)	8	98	(98 – 105)	8	ns
pH	5.1	(5.1 - 5.2)	9	4.9	(4.8 – 5.0)	13	**<0.001**
Alkalinity mmol L^{-1}	-0.011	(-0.014 - -0.009)	9	-0.017	(-0.019 - -0.012)	13	**0.017**
O_2 mg C L^{-1}	8.2	(7.4 - 8.9)	9	7.7	(7.3 - 8.5)	17	ns
TotN µg L^{-1}	379	(372 – 384)	9	383	(360 – 404)	13	ns
TotP µg L^{-1}	10	(9 – 11)	9	11	(10 – 12)	13	ns
DIC mg C L^{-1}	0.54	(0.46 - 0.87)	9	0.74	(0.65 - 1.22)	13	**0.045**
CO_2 eff. mg C m^{-2} d^{-1}	258	(191 – 449)	9	383	(324 – 664)	13	**0.038**
PP mg C m^{-2} d^{-1}	101	(56 – 121)	8	57	(44 – 81)	8	ns
CR mg C m^{-2} d^{-1}	99	(54 – 103)	8	79	(67 – 96)	8	ns
Chlorophyll*a* µg L^{-1}	5.4	(4.4 - 5.7)	8	5.1	(3.3 - 7.2)	8	ns
PB Tot.mm^3 L^{-1}	0.77	(0.51 - 1.70)	8	0.79	(0.60 - 1.57)	8	ns
Cryptophytes mm^3 L^{-1}	0.01	(0.00 - 0.02)	8	0.04	(0.02 - 0.07)	8	**0.028**
Dinophytes mm^3 L^{-1}	0.00	(0.00 - 0.01)	8	0.002	(0.00 - 0.04)	8	ns
Chrysophytes mm^3 L^{-1}	0.40	(0.20 -1.33)	8	0.166	(0.03 - 1.01)	8	ns
Diatomophytes mm^3 L^{-1}	0.00	(0.00 – 0.00)	8	0.00	(0.00 - 0.00)	8	ns
Raphidophytes mm^3 L^{-1}	0.00	(0.00 - 0.00)	8	0.03	(0.00 - 0.28)	8	ns
Chlorophytes mm^3 L^{-1}	0.33	(0.11 - 0.42)	8	0.255	(0.14 - 0.43)	8	ns
Cladocera, ind. L^{-1}	3.1	(0.7 - 17.0)	8	13.1	(6.2 - 22.9)	8	ns
Copepoda, ind. L^{-1}	15.7	(5.9 - 30.8)	8	18.9	(3.9 - 40.8)	8	ns
Perch density, ind ha^{-1}	657		1	1040		2	
Perch, 1st year growth, mm	69.4	(64.1 - 74.3)	44	61.4	(58.2 - 69.5)	17	**0.005**
Perch, 2nd year growth, mm	30.8	(23.6 - 38.7)	65	37.1	(33.9 - 41.2)	38	**<0.001**



Suppl. Fig. 1. Annual precipitation (mm) and mean air temperature (˚C) during the study years 1990-2009 at Lammi Biological Station, located at ca. 20 km south of the study lakes VK and AM.